Form PTO-1390	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER P20718
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/763625
INTERNATIONAL APPLICATION NO. PCT/JP99/04834	INTERNATIONAL FILING DATE 7 September 1999	PRIORITY DATE CLAIMED 7 September 1998

TITLE OF INVENTION

ANTIBODY FOR DETECTING POSSIBILITY OF ONSET OF BOVINE LEUKEMIA

APPLICANT(S) FOR DO/EO/US

Yoko AIDA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. _X_ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. _X_ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. _X_ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. _X_ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. _X_ is attached hereto (required only if not communicated by the International Bureau).
   b. _X_ has been communicated by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. _X_ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ___ are attached hereto (required only if not communicated by the International Bureau).
   b. ___ have been communicated by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ___ have not been made and will not be made.
8. ___ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. _X_ An oath or declaration of the inventor(s) (35 U.S.C. .371(c)(4)).
   "Unexecuted"
10. ___ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (U.S.C. 371(c)(5)).

Items 11 to 16 below concern other document(s) or information included:

11. Assignee: RIKEN of Saitama, JAPAN
12. ___ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. ___ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. _X_ A FIRST preliminary amendment.
    ___ A SECOND or SUBSEQUENT preliminary amendment.
15. ___ A substitute specification.
16. ___ A change of power of attorney and/or address letter.
17. ___ Figure of Drawing to be published_____
18. _X_ Other items or information:
    Cover Sheet and International Application as published in Japanese.
    PCT/RO/101-PCT Request(in Japanese).
    PCT/IB/301.
    PCT/IB/304.
    PCT/IB/308.
    PCT/IB/332.
    PCT/IPEA/408(in Japanese).
    PCT/IPEA/409(in Japanese).
    PCT/ISA/210(in English and Japanese).
    Cover Letter under 35 USC 371 and 1.494.
    Claim of Priority.

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/763625	INTERNATIONAL APPLICATION NO. PCT/JP99/04834	ATTORNEY'S DOCKET NUMBER P20718

19. ___ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search report has been prepared by the EPO or JPO. . . . . $ 860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482). . . . . $ 690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO(37 CFR 1.445(a)(2). . . . . $ 710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO. $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4). . . . . $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	RATE		
Total Claims	12 - 20 =	0	X $18.00	$0.00	
Independent Claims	4 - 3 =	1	X $80.00	$80.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$0.00	
			TOTAL OF ABOVE CALCULATIONS =	$940.00	
___ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$	
			SUBTOTAL =	$940.00	
Processing fee of $130.00 for furnishing the English translation later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					
Extension of Time fee in the amount of $					
			TOTAL NATIONAL FEE =	$940.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +					
			TOTAL FEES ENCLOSED =	$940.00	
			Amount to be refunded	$	
			Charged	$	

a. _X_ A check in the amount of $940.00 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees.

c. _X_ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0089.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO **CUSTOMER NO. 7055**
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P20718.A01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Yoko AIDA

Serial No : Not Yet Assigned (National Stage of PCT/JP99/04834)

Filed : Concurrently Herewith

For : ANTIBODY FOR DETECTING POSSIBILITY OF ONSET BOVINE LEUKEMIA

PRELIMINARY AMENDMENT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to calculation of the filing fees and the examination of the above-identified patent application on the merits, the Examiner is respectfully requested to amend the claims as follows:

IN THE CLAIMS

Please amend the claims as follows (a marked-up copy of the claim amendments is provided as an attachment to this Amendment):

5. (Amended-Clean Text) An agent for diagnosing a possibility of onset of bovine leukemia which comprises the monoclonal antibody according to claim 1.

6. (Amended-Clean Text) A method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of the monoclonal antibody according to claim 1.

Please add new claims 7-12 as follows:

---7. An agent for diagnosing a possibility of onset of bovine leukemia which comprises the monoclonal antibody according to claim 2.

8. An agent for diagnosing a possibility of onset of bovine leukemia which comprises the monoclonal antibody according to claim 3.

9. An agent for diagnosing a possibility of onset of bovine leukemia which comprises the monoclonal antibody according to claim 4.

10. A method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of the monoclonal antibody according to claim 2.

11. A method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of the monoclonal antibody according to claim 3.

12. A method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of the monoclonal antibody according to claim 4.---

REMARKS

By the above amendment, claims 5 and 6 have been amended and new claims 7-12 have been added to delete multiple dependency.

If there should be any questions, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,
Yoko AIDA

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March 6, 2001
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MARKED-UP COPY OF AMENDED CLAIMS

5. (Amended) An agent for diagnosing a possibility of onset of bovine leukemia which comprises the monoclonal antibody according to [any one of claims 1 to 4] claim 1.

6. (Amended) A method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of the monoclonal antibody according to [any one of claims 1 to 4] claim 1.

Specification

Antibody for detecting possibility of onset of bovine leukemia

Technical Field

The present invention relates to a monoclonal antibody which is used for judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV.

Background Art

The major histocompatibility antigens (MHC antigens) are molecules involved in self-nonself differentiation in the defense mechanism of the living body against infection. They are classified into Class I molecule composed of α chain and β 2M, and class II molecule composed of α chain and β chain. A groove for trapping an antigen peptide is present on the α 1 and α 2 domains, and also on the α 1 and β 1 domains. They are featured to have the T cell receptor recognize only a fragmented peptide trapped in the groove, thereby achieve cell death (cellular immunity) by CD8+ cells which have recognized the class I antigens, as well as induce mainly antibody production (humoral immunity) by CD4+ cells which have recognized the class II antigens.

The MHC genes constitute a gene group most full of polymorphism, and the locations of pockets, shapes, sizes, and properties of the peptide trapping grooves are different among haplotypes. It is considered that association conditions of the trapped fragment peptides may vary depending on these differences, which decide immune response and disease sensitivity of each individual. The correlation between the MHC haplotypes and a resistance to a disease (disease insusceptibility) or a possibility of the onset of a disease (disease susceptibility) has been reported, for example, as to human immune deficiency virus (HIV), human T cell leukemia virus (HTLV) and malaria.

As for the bovine MHC (BoLA) class II genes, the existence of DQA, DQB, DRA, DRB, DNA, DOB, DYA, and DYB genes has been estimated so far. DRB3, inter alia, which is one of the three genes (DRB1 to B3) identified on the DRB genetic locus,

has been known to encode a functional protein, and the existence of 73 alleles has been revealed so far. However, there is almost no report about correlation between bovine infectious diseases and the bovine MHC (BoLA) haplotypes.

In particular, as to the bovine leukemia virus (BLV), which has the gene pX that regulates virus proliferation in the same manner as the human immunodeficiency virus (HIV) and is a retrovirus most related to HTLV-I, a research group in the United States has reported its relationship with the bovine MHC (BoLA) haplotypes mainly focusing disease resistance; however, its relationship with possibility of onset of the leukemia has not been reported. The ratio of cattle infected by this virus (infection rate in Japan) is 10 to 20%, and 1 to 2% of the infected cattle develops extremely malignant endemic bovine leukemia to die after a long latent period of 10 to 15 years. Therefore, economic loss of stockbreeders caused by the virus is very serious. If a possibility of the onset of cattle after BLV infection can be evaluated by the analysis of bovine MHC (BoLA) haplotypes, it becomes possible to preliminarily select disease resistant cattle for bleeding beforehand, and it is expected that extremely safe cattle breeding can be continued.

The inventors of the present invention previously analyzed the structure of DRB gene locus among the bovine MHC (BoLA) class II genes, and reported the structures of DRB3 gene (BoLA-DRB3) and the gene product thereof (Biochem. Biophys. Res. Commun., 209, pp.981-988, 1995). The inventors further studied the function of the gene and found that a portion is present, whose amino acid sequence is distinctly different between cattle developing the leukemia and cattle not developing the disease, in the gene product from the second exon (β 1 domain) of BoLA-DRB3 showing particularly noticeable polymorphism. They also found that the amino acid substitutions directly correlated with disease susceptibility to BLV and disease resistance. Moreover, they found that, in judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV, a bovine individual, in which an amino acid sequence defined by the amino acid numbers 75 to 78 of the β 1 domain of the bovine MHC Class II DR β chain is Val-Asp-Thr-Tyr, can be judged to have a possibility of the onset of the leukemia, and they achieved the invention relating to the method (International Publication WO98/3680).

A monoclonal antibody (c143 monoclonal antibody) is known to react with a

tumor-associated antigen that is excessively expressed in BLV infected cells with progress of pathologic state of bovine leukemia ((a) Aida, Y. et al., Cancer Research, 52, pp.6463-6470, 1992; (b) Aida, Y. et al., Cancer Research, 53, pp.429-437, 1993). The aforementioned publications (a) (p.6469, the left column) and (b) (p.436, the left column) suggested that the tumor-associated antigen recognized by the aforementioned monoclonal antibody is related to an MHC Class II antigen. However, details of the reaction between the monoclonal antibody and the MHC Class II antigens have not been elucidated, and moreover, the structure of the epitope of the aforementioned monoclonal antibody has not been known so far.

Disclosure of the Invention

When the aforementioned method of judgment (WO98/3680) is carried out, it is necessary to collect a sample of a living bovine individual, amplify a desired gene, and then determine a base sequence of exon 2 gene of BoLA-DRB3, or carry out the PCR-RFLP method. The aforementioned publication discloses a primer set useful for the judging method; however, it is troublesome and time-consuming to carry out the aforementioned judging method for numbers of bovine individuals. Accordingly, it has been desired to develop a more simple method for judgment.

Therefore, an object of the present invention is to provide a means of simply and accurately judging a possibility of onset of leukemia in bovine individuals caused by bovine leukemia virus (BLV). More specifically, the object is to provide a means of accurately judging a possibility of onset of leukemia in bovine individuals caused by bovine leukemia virus without necessity of determination of a base sequence of exon 2 of the BoLA-DRB3.

The inventors of the present invention made intensive studies to achieve the foregoing objects. As a result, they found that bovine individuals having a gene, encoding β 1 domain of the MHC Class II DR β chain and attributable to a possibility of the onset of bovine leukemia, can be detected with a monoclonal antibody which reacts with a tumor-associated antigen excessively expressed in BLV infecting cells (the c143 monoclonal antibody), and that a possibility of the onset of leukemia can be judged with extremely high accuracy. The present invention was achieved on the basis of the aforementioned findings.

The present invention thus provides c143 monoclonal antibody which is used for detecting a bovine individual which has a possibility of onset of bovine leukemia; c143 monoclonal antibody which is used for detecting a gene encoding β 1 domain of the bovine MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable; and c143 monoclonal antibody which is used for detecting a bovine individual which has a gene encoding β 1 domain of the MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable.

In addition, there are provided a monoclonal antibody which is used for detecting a gene encoding β 1 domain of the bovine MHC Class II DR β chain to which a possibility of the onset of bovine leukemia is attributable, wherein the monoclonal antibody has substantially the same reactivity as c143 monoclonal antibody to bovine MHC Class II DR molecule to which a possibility of onset of bovine leukemia is attributable; a monoclonal antibody which is used for detecting a bovine individual having a gene encoding β 1 domain of the MHC Class II DR β chain to which a possibility of the onset of bovine leukemia is attributable, wherein the monoclonal antibody has substantially the same reactivity as c143 monoclonal antibody to bovine MHC Class II DR molecule to which a possibility of the onset of bovine leukemia is attributable; and a monoclonal antibody which is used for detecting a bovine individual which has a possibility of onset of bovine leukemia, wherein the monoclonal antibody has substantially the same reactivity as c143 monoclonal antibody to bovine MHC Class II DR molecule to which a possibility of onset of bovine leukemia is attributable.

According to another aspect of the present invention, there is provided an agent for diagnosing a possibility of the onset of bovine leukemia which comprises the aforementioned monoclonal antibody, preferably the aforementioned c143 monoclonal antibody. There are also provided a method for detecting a gene encoding β 1 domain of bovine MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable by means of c143 monoclonal antibody; a method for detecting a bovine individual having a gene encoding β 1 domain of MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable by means of c143 monoclonal antibody; and a method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of c143 monoclonal antibody.

According to still another aspect, there are provided a monoclonal antibody which is capable of detecting a gene encoding β 1 domain of bovine MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable; a monoclonal antibody which is capable of detecting a bovine individual having a gene encoding β 1 domain of MHC Class II DR β chain to which a possibility of the onset of bovine leukemia is attributable; and a monoclonal antibody which capable of detecting a bovine individual having a possibility of onset of bovine leukemia. A preferred example of the monoclonal antibody is c143 monoclonal antibody.

Best Mode for Carrying Out the Invention

Cattle to be applied with the method of the present invention are not particularly limited. The method may be applied to any sorts of cattle including dairy cattle, dairy and beef cattle, beef cattle, working cattle, working and beef cattle and the like, so long as they have a possibility of infection by leukemia virus BLV and have a possibility of developing the leukemia owing to the infection. More specifically, examples include Japanese cattle such as Japanese Black and Japanese Shorthorn, or breeds such as Holstein, Jersey, Hereford, Aberdeen Angus, and Friesian. However, breeds are not limited to these examples.

As the monoclonal antibody of the present invention, c143 monoclonal antibody can preferably be used. In addition to the c143 monoclonal antibody, monoclonal antibodies may also be used which have substantially the same reactivity as the c143 monoclonal antibody to bovine MHC Class II DR molecule to which a possibility of onset of bovine leukemia is attributable. The wording "MHC Class II DR molecule" used in the present specification means a molecule that contains a part or all of the MHC Class II DR α chain and a part or all of the MHC Class II DR β chain.

The monoclonal antibody having substantially the same reactivity as the c143 monoclonal antibody can easily be chosen by persons skilled in the art on the basis of criteria whether or not the monoclonal antibody gives a result of judgment similar to that obtained by the c143 monoclonal antibody when diagnosis is carried out in accordance with the method specifically described in Examples of the present specification. As such monoclonal antibodies, those derived from appropriate

mammals including mice, rats and rabbit can be used. The c143 monoclonal antibody (mouse, IgG2b) can easily be prepared by persons skilled in the art by a method described in literature (Aida, Y. et al., Cancer Res., 45, pp.1174-1180, 1985).

As for an amino acid sequence specified by the amino acid numbers of 75 to 78 of the β1 domain of the bovine MHC Class II DR β chain of a bovine individual, when the amino acid sequence (the amino acid numbers 75 to 78) is Val-Asp-Thr-Tyr in both of the alleles, it is known that the bovine individual has a high risk of onset of the leukemia when the individual has been already infected by the bovine leukemia virus BLV, or when the individual becomes infected by the virus (International Publication WO98/3680: The disclosures of the publication are incorporated herein as disclosures of the present specification.). Whilst when the amino acid sequences in the alleles are heterozygote of Val-Asp-Thr-Tyr (VDTY) and Val-Asp-Thr-Val (VDTV); heterozygote of Val-Asp-Thr-Tyr (VDTY) and Val-Asp-Arg-Val (VDRV); homozygote of Val-Asp-Thr-Val (VDTV); homozygote of Val-Asp-Arg-Val (VDRV); heterozygote of Val-Asp-Arg-Val (VDRV) and Val-Asp-Thr-Val (VDTV) or the like, the bovine individual has a very low possibility of onset of the leukemia even if the bovine individual has been already infected by the bovine leukemia virus BLV or when the individual becomes infected by the virus.

Although it is not intended to be bound by any specific theory, the monoclonal antibody of the present invention, preferably the c143 monoclonal antibody, binds to the bovine MHC Class II DR molecule, and has high reactivity when the molecule has Val-Asp-Thr-Tyr (the amino acid numbers 75 to 78) in the amino acid sequence of its β chain. Accordingly, a bovine individual wherein high reactivity of the monoclonal antibody of the present invention is observed has a gene that codes for Val-Asp-Thr-Tyr (the amino acid numbers 75 to 78) in β1 domain of MHC Class II DR β chain (a gene to which a possibility of the onset of bovine leukemia is attributable), and said individual has a high possibility of onset of bovine leukemia. The amino acid sequence of the β1 domain of the bovine MHC Class II DR β chain has been reported by Aida et al. (Aida, Y., et al., Biochem. Biophys. Res. Commun., 209, pp.981-988, 1995).

The method for detecting a gene that encodes the β1 domain of the bovine MHC Class II DR β chain, to which a possibility of onset of bovine leukemia is

attributable, is not particularly limited, and any methods may be applied so long as they can detect a binding between the monoclonal antibody and the antigen. For example, any detecting method available to persons skilled in the art can be applied, including fluorescent antibody method, flow cytometry, ELISA, immunohistological assay and the like. In order to facilitate the detection, a monoclonal antibody labeled with a fluorescent substance, radioisotope, avidin (or biotin) or the like can be used as the monoclonal antibody. Such labeling methods are well-known to persons skilled in the art, and any appropriate means can be applied.

In a preferred embodiment of the present invention, reactivity between the bovine MHC Class II DR molecule and the monoclonal antibody can be examined by using lymphocytes as samples which are separated or collected from a bovine individual. For example, peripheral lymphocytes can be also prepared from leukocytes by collecting peripheral blood from a bovine individual by using a syringe containing an anticoagulant, obtaining a leukocyte layer by centrifugation under the conditions of 4℃ and 3,000 rpm for 20 minutes, and then treating the layer by the method of Miyasaka et al. (Miyasaka, M. and Trnka, Z., Immunological Methods, Vol.3, pp.403-423, 1985, Academic Press, NY.).

When the monoclonal antibody of the present invention has high reactivity to the peripheral lymphocyte, the bovine individual is judged to have a possibility of onset of bovine leukemia. As the sample, a section of the lymph node, tumor tissues or the like may also be used. The degree of reactivity of the monoclonal antibody can be examined usually by preparing a control group or using a standard sample and the like. In addition, a gene encoding bovine MHC Class II DR molecule is amplified by the PCR method, and then reactivity of the monoclonal antibody to the gene product may be investigated.

The diagnostic agent of the present invention comprises the aforementioned monoclonal antibody as an active ingredient, and is used for judgment whether or not a bovine individual has a possibility of onset of bovine leukemia. In general, it is known that diagnostic agents comprising a monoclonal antibody can be formulated in various forms, and accordingly, the diagnostic agent of the present invention can be formulated in any appropriate forms. For example, the agent can be provided as preparations in a freeze-dried form or those in a liquid form or the like. The

diagnostic agent of the present invention can be prepared by using one or more kinds of appropriate additives for formulation depending on a form thereof. As the additives for formulation, for example, pH adjusting agents, dissolving aids, antiseptics, buffering agents, excipients and the like can be used. However, the additives are not limited to these examples.

Examples

The present invention will be explained more specifically by referring to examples. However, the scope of the present invention is not limited to the examples set out below.

1. Materials and Methods

Peripheral blood lymphocytes were fractioned from bovine individuals having a gene that codes for bovine MHC Class II DR β chain, to which resistance or sensitivity to onset of leukemia caused by BLV being attributable, and then mRNAs were extracted. cDNAs were synthesized with a reverse transcriptase by using an oligo (DT) primer and the mRNAs as templates. Then, using the resulting cDNAs as templates, cDNA clones containing the entire encoding region of DR β chain were isolated by the PCR method using the two primers:
5'-TGGCTCGAGCCTCTGCTGTTCTCCGGCAT-3' and
5'-TGGTCTAGAACTTCAGCTCAGGAGCCCTG-3'.
The primers used were designed to have XhoI and XbaI sites. The resulting PCR products (cDNA clones) were subcloned to a sequencing vector, and then the base sequences were determined to verify that desired genes were obtained.

As alleles of the gene encoding β 1 domain of MHC Class II DR β chain (hereinafter referred to as "BoLA-DRB3") to which resistance to leukemia caused by BLV is attributable, cDNAs of *0902, *0701, *1101, and *1401 were isolated. As the BoLA-DRB3 alleles responsible for sensitivity to the leukemia, cDNAs of *1501, *1601, *1302, and *1001 were isolated. Each cDNA was inserted into expression vector pME18Neo at XhoI and XbaI sites, and temporally co-transformed into COS1 cells or the 23CLN cells together with an expression vector previously isolated which was inserted with a cDNA clone containing the entire coding region of α chain (Aida, A.,

et al., Biochem. Biophys. Res. Commun., 204, pp.195-202, 1994). About 40 hours after the transformation, indirect immunofluorescence and flowcytometry were carried out using c143 monoclonal antibody to analyze reactivity.

2. Results

The c143 monoclonal antibody strongly reacted with the DR antigen-expressing cells introduced with the cDNA of BoLA-DRB3 gene responsible for sensitivity to onset of leukemia caused by BLV. Among them, the c143 monoclonal antibody had extremely strong reactivity to cells under expression which was introduced with *1601 cDNA, an allele most frequently found in cattle after the onset of leukemia. The results are shown in Table 1 set out below. In the table, * reactivity was classified into +: weak, ++: medium, and +++: strong on the basis of reactivity to c143 monoclonal antibody; **an allele encoding V as an amino acid residue of amino acid number 78 in DR β chain was judged as resistant to the onset of leukemia, whilst an allele encoding Y as disease susceptible; *** BoLA-DRB3 *1601 cDNA clone has already been isolated and referred to as NR1 (Aida, Y. et al., Biochem. Biophys. Res. Commun., 209, pp.981-988, 1995).

Table 1

cDNA of α chain/cDNA of β chain	Amino acid residue of amino acid number 78 of DR β chain: V or Y**	Reactivity to c143 antibody*
MR1 / BoLA-DRB3*0902	V	+
MR1 / BoLA-DRB3*0701	V	+
MR1 / BoLA-DRB3*1101	V	+
MR1 / BoLA-DRB3*1401	V	+
MR1 / BoLA-DRB3*1501	Y	++
MR1 / BoLA-DRB3*1601 (NR1)***	Y	+++
MR1 / BoLA-DRB3*1302	Y	++
MR1 / BoLA-DRB3*1001	Y	++

Industrial Applicability

By using the monoclonal antibody of the present invention, a possibility of onset of bovine leukemia virus (BLV) of bovine individuals can be conveniently and accurately judged.

What is claimed is:

1. A c143 monoclonal antibody which is used for detecting a bovine individual which has a possibility of onset of bovine leukemia.

2. A c143 monoclonal antibody which is used for detecting a gene encoding β 1 domain of bovine MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable.

3. A monoclonal antibody which is used for detecting a bovine individual having a possibility of onset of bovine leukemia, wherein the monoclonal antibody has substantially the same reactivity as a c143 monoclonal antibody to a bovine MHC Class II DR molecule to which a possibility of onset of bovine leukemia is attributable.

4. A monoclonal antibody which is used for detecting a gene encoding β 1 domain of bovine MHC Class II DR β chain to which a possibility of onset of bovine leukemia is attributable, wherein the monoclonal antibody has substantially the same reactivity as a c143 monoclonal antibody to a bovine MHC Class II DR molecule.

5. An agent for diagnosing a possibility of onset of bovine leukemia which comprises the monoclonal antibody according to any one of claims 1 to 4.

6. A method for detecting a bovine individual which has a possibility of onset of bovine leukemia by means of the monoclonal antibody according to any one of claims 1 to 4.

Abstract

A c143 monoclonal antibody which is used for detecting a bovine individual having a possibility of onset of bovine leukemia; a monoclonal antibody which is used for detecting a bovine individual having a possibility of onset of bovine leukemia, wherein the monoclonal antibody has the substantially same reactivity as the c143 monoclonal antibody to a bovine MHC Class II DR molecule to which a possibility of onset of bovine leukemia is attributable; and an agent for diagnosing a possibility of onset of bovine leukemia which comprises the aforementioned monoclonal antibody. Bovine individuals having a possibility of onset of bovine leukemia can be conveniently and accurately detected.

P20718 FA1502M/US

Case # P20718

Declaration and Power of Attorney For Utility or Design Patent Application
特許出願宣言書

Japanese Language Declaration

私は、下欄に氏名を記載した発明者として、以下のとおり宣言する：

As a below named inventor, I hereby declare that:

私の住所、郵便の宛先および国籍は、下欄に氏名に続いて記載したとおりであり、

My residence, post office address and citizenship are as stated below next to my name.

名称の発明に関し、請求の範囲に記載した特許を求める主題の本来の、最初にして唯一の発明者である（一人の氏名のみが下欄に記載されている場合）か、もしくは本来の、最初にして共同の発明者である（複数の氏名が下欄に記載されている場合）と信じ、

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

ANTIBODY FOR DETECTING POSSIBILITY OF ONSET OF BOVINE LEUKEMIA

上記発明の明細書（下記の欄でX印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ _____年_____月_____日に提出され、
米国出願番号 _____ とし、
（該当する場合）_____年_____月_____日に訂正されました。又は、

☒ was filed on 7/SEPTEMBER/1999 as
United States Application Number 09/763,625
and was amended on 6/March/2001 (if applicable) or,

特許協定条約国際出願番号 _____ とし、
（該当する場合）_____年_____月_____日に訂正されました。

PCT International Application Number PCT/JP99/04834
and was amended on _____ (if applicable).

私は、前記のとおり補正した請求の範囲を含む前記明細書の内容を検討し、理解したことを陳述する。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第１条第56項に定義されるとおり、特許資格の有無について重要な情報を開示すべき義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

私は合衆国法典第35部第119条(a－d)項又は第365条(b)項に基づく、下記の外国特許出願又は発明者証出願、或いは第365条(a)項に基づく、少なくても米国以外の1ケ国を指名したPCT国際出願の外国優先権を主張し、更に優先権の主張に係わる基礎出願の出願日前の出願日を有する外国特許出願、又は発明者証出願或るいはPCT国際出願を以下に"なし"の箱に印をつけることにより明記する：

I hereby claim foreign priority under Title 35, United States Code §119(a-d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States, listed below. I have also identified below, by checking the "No" box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior foreign applications 先の外国出願 (Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願の年月日)	Priority claimed 優先権の主張 Yes あり	No なし
10/252128	JAPAN	7/SEPTEMBER/1998	☒	☐
			☐	☐

☐ その他の外国特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

Letter Version

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私は、合衆国法典第35部第119条(e)項に基づく、下記の合衆国仮特許出願の利益を主張する。

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Day/Month/Year Filed) 出願の年月日
(Application No.) (出願番号)	(Day/Month/Year Filed) 出願の年月日
(Application No.) (出願番号)	(Day/Month/Year Filed) 出願の年月日

☐ その他の合衆国仮特許出願番号は別紙の追補優先権欄にて記載する。

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

私は、合衆国法典第35部第120条に基づく下記の合衆国特許出願、又は第365条(c)項に基づく合衆国を指名したPCT国際出願の利益を主張し、本願の請求の範囲各項に記載の主題が合衆国法典第35部第112条第1項規定の態様で、先の合衆国特許出願又はPCT国際出願に開示されていない限度において、先の出願の出願日と本願の国内出願日又はPCT国際出願日の間に有効となった連邦規則法典第37部第 1 章第56条に記載の特許要件に所要の情報を開示すべき義務を有することを認める。

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)	(現況) (特許済み、係属中　放棄済み)	(Status) (patented, pending, abandoned)
(Application No.) (出願番号)	(Day/Month/Year Filed) (出願の年月日)	(現況) (特許済み、係属中　放棄済み)	(Status) (patented, pending, abandoned)

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☐ Additional U.S. or international application numbers are listed on a supplemental priority sheet attached hereto.

私は、ここに自己の知識にもとずいて行った陳述がすべて真実であり、自己の有する情報および信ずるところに従って行った陳述が真実であると信じ、さらに故意に虚偽の陳述等を行った場合、合衆国法典第18部第1001条により、罰金もしくは禁錮に処せられるか、またはこれらの刑が併科され、またかかる故意による虚偽による陳述が本願ないし本願に対して付与される特許の有効性を損なうことがあることを認識して、以上の陳述を行ったことを宣言する。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

私、下記署名者は、ここに記載の米国弁護士または代理人に本出願に関し特許商標庁にて取られるいかなる行為に関して、同米国弁護士又は代理人が、私に直接連絡なしに私の外国弁護士或るいは法人代表者からの指示を受け取り、それに従うようここに委任する。この指示を出す者が変更の場合には、ここに記載の米国弁護士又は代理人にその旨通知される。

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from either his foreign patent agent or corporate representative, if any, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Case # P20718

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委任状： 私は、下記発明者として、下記に明記された顧客番号を伴う以下の弁護士又は、代理人をここに選任し、本順の手続きを遂行すること並びにこれに関する一切の行為を特許商標庁に対して行うことを委任する。そして全ての通信はこの顧客番号宛に発送される。

顧客番号 7055

現在選任された弁護士は下記の通りである。

POWER OF ATTORNEY: As a named inventor, I hereby appoint the attorney(s) and/or agent(s) associated with the Customer Number provided below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

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（第六またはそれ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for third and subsequent joint inventors.)